

CLAIMS

1. A chimaeric phage having a coat comprising a mixture of proteins, said mixture comprising a fusion protein wherein a proteinaceous molecule is fused to a functional form of a phage coat protein, said mixture further comprising a mutant form of said phage coat protein,
5 wherein said mutant form is characterized in that a phage, comprising no wild type phage coat protein from which said mutant form is derived and having a coat comprising said mutant form and no copies of said functional form, is less infectious than a phage, comprising no wild type phage coat protein from which said mutant form is derived and having a coat comprising said mutant form and at least one copy of said functional form.
- 15 2. A chimaeric phage according to claim 1, wherein said phage coat protein is the g3 protein.
3. A chimaeric phage according to claim 2, wherein said mutant form comprises a mutation in the D1 and/or the D2
20 region of said g3 protein.
4. A chimaeric phage according to claim 3, wherein said mutation comprises a deletion of substantially all of said D1 and said D2 region of said g3 protein.
- 25 5. A chimaeric phage according to any one of claims 1-4 comprising a nucleic acid encoding said fusion protein.
6. A chimaeric phage according to any one of claims 1-5,
30 wherein said chimaeric phage is derived from a M13, M13K07, VCSM13 or R408 phage.

7. A chimaeric phage according to any one of claims 1-6, wherein said proteinaceous molecule comprises a peptide, a protein or a part, analogue or derivative thereof.

5 8. A chimaeric phage according to any one of claims 1-6, wherein said proteinaceous molecule comprises an antibody, a Fab fragment, a single chain Fv fragment, a variable region, a CDR region, an immunoglobulin or a functional part thereof.

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9. A chimaeric phage having a coat comprising a mixture of proteins, said mixture comprising a fusion protein wherein a proteinaceous molecule is fused to a phage coat protein, or to a fragment or derivative thereof, and 15 wherein said fusion protein is functional so as to render the chimaeric phage infectious, said mixture further comprising a mutant form of said phage coat protein, wherein said mutant form is characterized in that a phage, comprising no wild type phage coat protein from which said 20 mutant form is derived and carrying said mutant form and no copies of said fusion protein, is less infectious than a phage, comprising no wild type phage coat protein from which said mutant form is derived and carrying in addition to said mutant form at least one copy of said fusion 25 protein.

10. A chimaeric phage according to claim 9, wherein said mutant form is characterized in that a phage, comprising no wild type phage coat protein from which said mutant 30 form is derived and carrying said mutant form and no copies of said fusion protein is non-infectious.

11. A chimaeric phage according to any one of claims 1-10, wherein said mutant form is further characterized in 35 that a phage, having a coat comprising said mutant form in

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the presence or absence of copies of said functional form, is stable.

12. An infectious phage containing at least one copy of a
5 mutant form of a phage coat protein, wherein said mutant form has lost the ability to mediate infection of a natural host by said infectious phage.

13. A phage collection comprising a chimaeric phage
10 according to any one of claims 1-11 or an infectious phage according to claim 12.

14. A phage collection according to claim 13, wherein said phage collection is a phage display library.

15. A phage collection consisting essentially of chimaeric phages according to any one of claims 1-11 or of infectious phages according to claim 12.

20 16. A method for producing a phage particle comprising the steps of:

- providing a host cell with a first nucleic acid encoding a fusion protein, said fusion protein comprising a proteinaceous molecule fused to a functional form of a phage coat protein,
- providing said host cell with a second nucleic acid encoding a mutant form of said phage coat protein, said mutant form being characterized in that a phage, comprising no wild type phage coat protein from which said mutant form is derived and having a coat comprising said mutant form and no copies of said functional form, is less infectious than a phage comprising no wild type phage coat protein from which said mutant form is derived and having a coat comprising at least one copy of said functional form, and wherein said host cell comprises an additional nucleic acid sequence encoding at least all other proteins

or functional equivalents thereof, that are essential for the assembly of said phage particle in said host cell, and - culturing said host cell to allow assembly of said phage particle.

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17. A method according to claim 16, wherein expression of said fusion protein and/or said mutant form is regulatable by altering the culturing conditions of said host cell.

10 18. A method according to claim 16 or 17, wherein expression of said fusion protein and/or said mutant form is under the control of a regulatable promoter.

15 19. A method according to claim 18, wherein said regulatable promoter comprises the AraC/BAD promoter or a functional equivalent thereof.

20 20. A method to any one of claims 16-19, wherein said additional nucleic acid sequence is provided by a helper phage to said host cell.

21. A method according to claim 20, wherein said helper phage comprises said second nucleic acid.

25 22. A method according to any one of claims 16-20, wherein said fusion protein and said mutant form are encoded by separate nucleic acids each comprising a unique selection marker.

30 23. A method according to claim 22, wherein said separate nucleic acids each comprises a unique origin of replication.

35 24. A method according to claim 22 or 23, wherein said separate nucleic acids each comprises codons that

essentially do not render a homologous recombination event between said separate nucleic acids.

25. A method according to any one of claims 16-24,
5 wherein said phage particle is a chimaeric phage according to any one of claims 1-11 or an infectious phage according to claim 12.

26. A helper phage comprising nucleic acid encoding phage
10 proteins or functional equivalents thereof that are essential for the assembly of said helper phage, said nucleic acid further encoding a mutant form of a phage coat protein, wherein said mutant form is characterized in that a phage, comprising no wild type phage coat protein
15 from which said mutant form is derived and having a coat comprising said mutant form and no copies of a functional form of said phage coat protein, is less infectious than a phage, comprising no wild type phage coat protein from which said mutant form is derived and having a coat
20 comprising at least one copy of said functional form, wherein said functional form is characterized in that it renders a phage particle carrying said functional form in its coat infectious, and wherein said helper phage does not comprise nucleic acid encoding said functional form.
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27. A helper phage according to claim 26, wherein said phage coat protein is the g3 protein.

28. A helper phage according to claim 27, wherein said mutant form comprises a mutation in the D1 and/or the D2 region of said g3 protein.
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29. A helper phage according to claim 28, wherein said mutation comprises a deletion of substantially all of said
35 D1 and said D2 region of said g3 protein.

30. A helper phage according to any one of claims 26-29, wherein said mutant form is further characterized in that a phage, having a coat comprising said mutant form in the presence or absence of a copy of said functional forms, is
5 stable.

31. A method for producing a helper phage comprising the steps of:

- providing a host cell with a first nucleic acid encoding a functional form of a phage coat protein,
- providing said host cell with a second nucleic acid encoding a mutant form of said phage coat protein, wherein said mutant form is characterized in that a phage, comprising no wild type phage coat protein from which said mutant form is derived and having a coat comprising said mutant form and no copies of said functional form, is less infectious than a phage, comprising no wild type phage coat protein from which said mutant form is derived and having a coat comprising at least one copy of said functional form, wherein said host cell comprises an additional nucleic acid sequence encoding at least all other proteins or functional equivalents thereof that are essential for the assembly of said helper phage in said host cell, and
- 25 - culturing said host cell to allow assembly of said helper phage.

32. A method according to claim 31, wherein said other proteins or functional equivalents thereof that are essential for the assembly of said helper phage in said host cell are encoded by said second nucleic acid.

33. A method according to claim 31 or 32, wherein expression of said functional form and/or said mutant form is regulatable by altering the culturing conditions of said host cell.

34. A method according to any one of claims 31-33,
wherein expression of said functional form and/or said
mutant form is under the control of a regulatable
5 promoter.

35. A method according to claim 34, wherein said
regulatable promoter comprises the AraC/BAD promoter or a
functional equivalent thereof.
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36. A method according to any one of claims 31-35,
wherein said phage coat protein is the g3 protein.
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37. A method according to claim 36, wherein said mutant
form comprises a mutation in the D1 and/or the D2 region
of said g3 protein.
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38. A method according to claim 37, wherein said mutation
comprises a deletion of substantially all of said D1 and
said D2 region of said g3 protein.
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39. A method according to any one of claims 31-38,
wherein said first nucleic acid and said second nucleic
acid each comprises a unique selection marker.
40. A method according to any one of claims 31-39,
wherein said first nucleic acid and said second nucleic
acid each comprises a unique origin of replication.
30 41. A method according to any one of claims 31-40,
wherein said first nucleic acid and said second nucleic
acid comprise codons that essentially do not render a
homologous recombination event between said first nucleic
acid and said second nucleic acid.
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42. A method according to any one of claims 31-41, wherein said helper phage is a helper phage according to any one of claims 26-30.

5 43. A method for the enrichment of a first binding pair member in a repertoire of first binding pair members selected from the group consisting of an antibody, an antibody fragment, a single chain Fv fragment, a Fab fragment, a variable region, a CDR region, an
10 immunoglobulin or a functional part thereof, said first binding pair member being specific for a second binding pair member, comprising the steps of
- contacting a phage collection according to any one of claims 13-15 with material comprising said second
15 binding pair member under conditions allowing specific binding,
- removing non-specific binders, and
- recovering specific binders, said specific binders comprising said first binding pair member.
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44. A method according to claim 43 comprising the additional steps of
- recovering from a phage a DNA sequence encoding said first specific binding pair member,
25 - subcloning said DNA sequence in a suitable expression vector, and
- expressing said DNA sequence in a suitable host, and
- culturing said suitable host under conditions
30 whereby said first specific binding pair member is produced.

45. A nucleic acid molecule comprising a sequence encoding a mutant form of a phage coat protein, said
35 mutant form being characterized in that a phage, comprising no wild type phage coat protein from which said

mutant form is derived and having a coat comprising said mutant form and no functional form of said phage coat protein, is less infectious than a phage, comprising no wild type phage coat protein from which said mutant form 5 is derived and having a coat comprising said mutant form and at least one copy of said functional form of said phage coat protein, wherein said functional form is characterized in that it renders a phage carrying said functional form in its coat infectious.